77-Plat

Somatic Sodium Channels Account for Second Phase of Action Potential Upstroke in Soma of Layer 5 pyramidal Cells

Andreas Neef[1,2], Fred Wolf[2,1], Michael J. Gutnick[3], Ilya Fleidervish[4].
[1]Bernstein Centre for Computational Neuroscience, Göttingen, Germany, [2]Max-Plack-Institute for Dynamics and Self-Organization, Göttingen, Germany, [3]Koret School of Veterinary Medicine, Hebrew University of Jerusalem, Rehovot, Israel, [4]Department of Physiology, Faculty of Health Sciences, Ben-Gurion University of the Negev, Beer Sheva, Israel.

Mechanisms of action potential (AP) generation in neocortical pyramidal cells have been the focus of intense experimental and theoretical research over the last several decades. It has proven very difficult, however, to arrive at a consensus model which can satisfactorily account for all of its features. One of the still unresolved issues is lack of accurate description of Na^+ channel kinetics in different neuronal compartments. Here, we measured kinetics of somatic Na^+ channels using high temporal resolution (5-10 kHz, −3dB, low pass four-pole Bessel filter) cell-attached recordings from layer 5 pyramidal neurons in neocortical slices. The data were described by fitting different Markov models with differential evolution fit algorithms. The limited speed of voltage steps and the effect of current filtering were accounted for in the fit procedure. Hodgkin-Huxley-type models which assumed a number of independent activation gates were not the optimal description of the experimentally recorded currents. Activation kinetics was best described by Markov models with two sequentially activating gates, while inactivation was best described as a process that runs in parallel to activation. The best model described the channel data well enough to allow quantitative prediction of the somatic Na^+ current during the somatic spike. To this end the AP waveform recorded in current clamp in the same preparation, was used to drive Na^+ channels in the model. The resulting simulated current matched the second phase of the AP upstroke in the phase plot (dV/dt vs V). This is consistent with the long standing idea that somatic Na^+ channels are the main current sink during this second phase of the AP upstroke but contribute little to its initial phase.

Supported by the GIF and the BMBF (BCCN, Goettingen).

78-Plat

Slow Inactivation of Nav Channels Described by Composite Movements of Voltage Sensors in Domains I, II and III

Jonathan R. Silva, Steve A.N. Golstein.
University of Chicago, Chicago, IL, USA.

Slow inactivation (SI) of voltage-gated sodium (Na_V) channels is a primary determinant of their 'availability' and therefore the excitability of muscles and nerves. This process has been difficult to describe because it is a multi-timescale phenomenon that can be perturbed by mutations at many channel locales including the S4 voltage sensors (VS). Here, we compare ionic and gating currents during SI with changes in fluorescence from probes conjugated to the VS in each of the four domains (DI-DIV) of the rat skeletal muscle isoform ($Na_V1.4$). We observe that onset and recovery of SI can be approximated by three time constants of ~1, 10 and 100 s and that these temporal components are reflected in domain-specific fluorescence immobilization. The kinetics of immobilization of DI and DII correlate with the 10 s component and are potently inhibited by TTX. Immobilization of DIII reflects both the 1 and 100 s components and enhancing the speed and magnitude of SI via a mutation that removes fast inactivation (IQM) has the same effects on DIII immobilization. Limited immobilization observed for DIV shows no correlation with SI. Rapid gating is a hallmark of Na_V channels and results from movements of VSs over ms. This study links slow, differential movements of VSs in DI, DII and DIII with the time constants required to model SI kinetics over multiple time domains. The findings offer a basis for understanding the effects on SI of previously reported disease-associated and experimental mutations in the pore, inactivation lid and VSs.

79-Plat

Individual Residues of μ-Conotoxin PIIIA Appear to Show Distinct Electrostatic Interactions with Ions in the Conduction Pathway of Sodium Channels

Jeffrey R. McArthur, Gurpreet Singh, Denis McMaster, D. Peter Tieleman, Robert J. French.
University of Calgary, Calgary, AB, Canada.

μ-Conotoxins are highly basic peptides which block the ion conduction pathway of voltage-gated sodium (NaV) channels by a combination of steric and electrostatic actions. Toxins, which block NaV channels at site one (saxitoxin derivatives, tetrodotoxin, and μ-conotoxins), show a similar voltage dependence of binding (apparent valence, zδ~0.6) despite a wide range of nominal net charges (−1 to +7), suggesting a common mechanism underlying the voltage dependence. For PIIIA, single-channel studies using lipid bilayers reveal three different positions, R12, R14 and K17, at which neutral substitutions enable varying amplitudes of current to flow through toxin-bound channels. Also, when individual PIIIA residues are replaced, with addition or subtraction of one charge, the voltage dependence of block changes to various degrees depending on the position on the surface of the toxin. The largest changes in zδ correspond to residues believed to enter most deeply into the channel pore, while residues facing the external solution have little effect. Residues in intermediate positions affect the voltage dependence inversely with their estimated distance from the selectivity filter. The assembled toxin voltage dependencies are most easily explained if the voltage dependence reflects toxin-induced displacement of a Na^+ ion within in the pore, rather than substantial movement of toxin charge through the applied voltage. Calculations using the Applied Poisson-Boltzman Solver (APBS), with a model of the sodium channel outer vestibule, suggest that docking of different PIIIA derivatives changes the electrostatic profile in the pore, depending on the location of the charge change on the toxin. Those changes are generally consistent with both the range of voltage dependencies observed, and the occurrence of partial conductances, when certain derivatives (such as R12A, R14A, and K17A), are bound to single channels.

80-Plat

Biochemical and Biophysical Characterization of a Sodium Channel Pore Protein

David Shaya[1], Mohamed Kreir[2], Justus Hammon[1], Andrea Brüggemann[2], Daniel L. Minor[1,3].
[1]UCSF, San Francisco, CA, USA, [2]Nanion Technologies, Munich, Germany, [3]Lawrence Berkeley National Laboratory, Berkeley, CA, USA.

Voltage gated sodium channels (NaVs) are large polytopic membrane proteins involved in the generation of action potentials in excitable cells. Mutations in these channels are involved in arrhythmias, pain disorders, and some forms of hereditary epilepsy. Recently, single subunit NaV homologs have been identified in a large variety of marine bacteria. Sequence conservation at key points along the ion conducting path between the eukaryotic and prokaryotic NaVs suggests that the prokaryotic channels can serve as an excellent model for understanding the basic aspects of NaVs function and the effects of disease mutations that lead to channel malfunction.

Using a protein engineering approach based on the likelihood that the voltage-sensor and pore domains are independently folded domains, we dissected the voltage sensor from the ion conducting part of bacterial ion channels to create a stand-alone pore protein. The 'NaV pore' expresses a stable protein that can be isolated and purified to homogeneity. Extensive biochemical and biophysical characterization demonstrate that the 'NaV pore' self-assembles as tetramers that show the characteristics of an alpha-helical membrane protein. Furthermore, the 'NaV pore' conducts sodium currents when incorporated into lipid vesicles. Thus, the 'NaV pore' represents an active ion channel that provides an excellent model system for the study of the factors that govern sodium selectivity and permeability.

81-Plat

Mapping the Structure of the Cardiac Voltage-Gated Sodium Channel (Nav1.5) C-Terminus: Implications of An Lqt3 Mutant

Ian W. Glaaser, Jeremiah D. Osteen, Akil Puckerin, Robert S. Kass.
Columbia University, New York, NY, USA.

The cardiac voltage-gated sodium channel(Nav1.5) underlies the upstroke of the cardiac action potential and can contribute to action potential duration. Defects in sodium channel inactivation, for which the carboxy terminus(C-T) plays an important role, are linked to cardiac arrhythmia. Studies of the C-T identify four proximal helices that form an EF hand motif and two predicted distal helices whose location relative to the EF hand motif are unknown. We used transition metal ion FRET to measure intramolecular interactions within the C-T to locate the predicted distal helix(H6). A single intrinsic tryptophan in the 1st C-T helix(H1), and Ni2+ coordinated by engineered di-histidine residues were used as the fluorescence donor and acceptor, respectively. Initial experiments confirmed the validity of the use of Trp as the donor and measured the distance between the Trp and a di-histidine on the 4th helix consistent with the NaV1.5 EF NMR (16.7 ± 0.3 vs. 16.8Å). Next, a distance between the Trp and a di-histidine on the middle of H6 was measured, demonstrating this interaction for the first time in cis. Introduction of an LQT3 mutation, S1904L, previously shown to disrupt inactivation, in the H6 di-Histidine background increased the distance such that there was no FRET. This indicated that the di-Histidine had moved >5 Å. The change in distance correlates with disruption of inactivation, suggesting that the H6 interaction with the EF hand domain may be necessary for proper inactivation. Finally, we were able to measure distances for additional sites on H6 providing information about the orientation and distance of H6 relative to the EF hands. These data provide a structural framework within which mutational effects can be examined and provide a structural correlate to altered function in an LQT3 mutant channel.